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Research report

Impact of high and low anxiety trait on object habituation and discrimination: Evidence from selected lines of Japanese quail

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HIGHLIGHTS

- High anxiety trait has no effect on object habituation in Japanese quail.
- High anxiety trait enhanced object discrimination in Japanese quail.
- High anxiety trait is associated with accurate processing of environmental cues.

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ABSTRACT

Compared to rodents, the relationship between anxiety and cognitive performances has been less studied in birds. Yet, birds are frequently exposed to stimulations that constitute a potential source of anxiety and can affect their adaptation to their living conditions. The present study was aimed at evaluating, in birds, the relationship between levels of anxiety and object habituation and discrimination with the use of Japanese quail lines divergently selected for a fear response, tonic immobility. Previous studies demonstrated that the selection programme has modified the general anxiety trait of the birds. The task consisted in 4 daily sessions of 8 successive presentations of the same object in the home cage of the quail in order to habituate each bird to the object. The observation that both quail with a high and a low anxiety trait progressively spent more time close to the object indicated that habituation occurred. Dishabituation was assessed during a single session of 8 presentations of a novel object. Only quail with a high anxiety trait exhibited significant discrimination. They spent significantly less time close to the novel object than to the habituated object. It is hypothesised that a high anxiety trait is associated with a more accurate processing of environmental cues or events resulting in better discriminative performances.

1. Introduction

The emotional state of a subject modulates its learning and memory abilities [1,2]. Low to moderate emotional arousal can facilitate learning and memory [3]. This phenomenon is particularly important in humans and more generally for animals to identify, store relevant information from their environment and adapt their behaviour accordingly. In contrast, high levels of anxiety generally impair cognitive abilities including learning and memory. Rodent models that are characterised by different levels of anxiety have largely contributed to identify the relationship between emotional arousal or anxiety and learning and memory. Studies with rats bred for high and low levels of anxiety [4–6] and strains of inbred mice differing in anxiety levels [7–9] have confirmed a close correlation between anxiety and cognition.

In the wild or in captive conditions, birds are frequently exposed to emotionally laden events that can modulate their learning and memory abilities and their adaptation to their living environment. However, compared to rodents, the influence of high or low level of anxiety or emotional arousal on cognitive performances in birds has been less studied. The present study was aimed at evaluating, in birds, the relationship between levels of anxiety and cognitive

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performance with the use of selected lines of Japanese quail characterised by different innate anxiety levels. Two lines of Japanese quail divergently selected for over 36 generations for their tonic immobility duration [10], a standard and robust measure of bird fearfulness, provide a valuable biological model for investigating whether emotional reactivity modulates cognitive performance in birds. One line is selected for its long tonic immobility duration (LTI line), and the other for short tonic immobility durations (STI line). Previous studies demonstrated that animals from the LTI line not only exhibit long tonic immobility durations but also show increased behavioural inhibition when exploring a novel environment during an open field test. LTI quail exhibit more reluctance to enter into a new environment, a longer latency to approach novel food and are more disturbed by the sudden introduction of a frightening stimulus into their home cage [11,12]. Interestingly, we recently showed that anxiolytic treatment could alleviate behavioural differences between the two lines in the open field test [13]. Physiological studies also identified significant differences in corticosterone response to restraint between these lines [14–16] and investigations on heart rate variability revealed that both basal level of the autonomic nervous system and level induced by an acoustic stimulus were different between the two lines [17,18]. Altogether, these findings have led to the assumption that selection for tonic immobility generally affects the emotional reactivity or anxiety levels of the quail.

In the present study, LTI and STI Japanese quail were submitted to a habituation–dishabituation procedure in order to test possible differences in discriminative performance between the two lines. This procedure was chosen because previous experiments provided evidence for its pertinence to test memory performance on the basis of novelty detection in many species including birds [19–25]. The experiment consisted in 4 daily sessions of 8 successive presentations of the same object in the home cage of the quail in order to habituate each bird to the object. Dishabituation was examined during a single session of 8 presentations of a novel object. Cognition, broadly defined, includes perception, learning, memory and decision making processes [26]. Most of these processes can affect habituation–dishabituation performances. Thus, the use of this basic procedure of habituation–dishabituation is a way to examine possible differences in cognitive performances between the two lines of birds.

2. Materials and methods

2.1. Animals

Twenty-three male quails from the 36th generation of 2 lines of Japanese quail genetically selected for their short ($N = 12$ STI) or long ($N = 11$ LTI) durations of tonic immobility [10] were used in the present study. These lines of quail are selected and maintained at the Pôle d'Expérimentation Avicole de Tours (UE PEAT, INRA, Nouzilly, France) where the experiment was carried out. The 2 selected lines were hatched and bred under the same conditions. On the day of hatching, chicks were wing-banded and transferred to communal floor pens for 4 weeks. From the age of 4 weeks until the start of the experiment quail were housed in collective cages (100 cm × 85 cm × 20 cm) and reared under a 12:12 h light dark schedule (light on at 0800 h). Sex and lines were separated once quail were sexually mature at the age of 6 weeks. This separation of lines is particularly important to limit the possible spreading of fearfulness from high fearful birds (LTI lines) to low fearful birds (STI lines). Indeed, in laying hens, fearful individuals may cause other hens to become more fearful [27]. For example, it has been shown that housing birds of a fearful line together with birds from a non-fearful line, led to increased fearfulness in the birds of the non-fearful line [28]. Using LTI and STI lines of Japanese quails it was also previously shown that the fearfulness of chicks adopted by LTI mothers was higher than that of chicks adopted by STI mothers [29]. In rodents [30,31] but also in avian species [32,33] social transmission of fear and early exposure to stress can have life-long effects on behaviour, physiology and brain development. Nevertheless, to minimise any effects of rearing group, 3 days before the start of the experiment, LTI and STI quails were singly housed in the same animal room and all birds were individually tested during the habituation dishabituation procedure (see below).

Unless otherwise specified, food and water were freely available at all times. Animal care procedures were conducted in accordance with the guidelines set by the European Communities Council Directive (86/609/EEC) and French legislation on animal research.

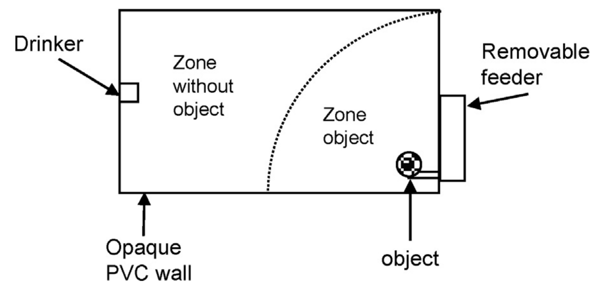

Fig. 1. Diagram of an experimental cage viewed from the top showing the site at which the object was introduced in the cage as well as the different zones used for behavioural analysis.

2.2. Testing apparatus and procedure

The testing apparatus has been previously described in detail and the procedure of testing habituation has been adapted from [34,35]. Animals were transferred to a testing room 3 days before testing. They were housed individually in PVC cages (42 cm × 24 cm × 25 cm, Fig. 1) with wood-shavings on the floor and a transparent PVC roof so that each quail could be observed with a video camera. The arrangement of the cages in the room and their design allowed to easy removing of the feed trough, with minimal disturbance for the quail (i.e. with the experimenter out of sight of the birds). Social isolation of the birds was reduced since the quail could hear other quail in the same room. The room was maintained at $22 \pm 2\,^{\circ}$C under a 12:12 h light dark schedule (light on at 0800 h).

2.2.1. Habituation

During 4 consecutive days, quail were submitted daily to 8 presentations of the same object. Each day, before the beginning of the session of 8 presentations, the food trough of every cage was removed for 2 h. At the end of this 2 h-period, the food trough was gently replaced in the cage for 2 min with an object attached to it. After this 2-min period, the food trough was removed for 8 min until the next presentation of the same object. Eight minutes after the end of the last presentation of the object, the food trough was replaced in the cage without any object attached to it. The amount of time spent in the zone close to the object was recorded because this parameter has previously been shown to be relevant to assess object habituation in Japanese quail [34,35]. The object attached to the food trough during habituation was a white plastic ball of 40 mm in diameter. Twenty dots (2 mm in diameter) of red, blue, and green colours had been drawn on the surface of the ball.

2.2.2. Dishabituation

On the 5th day, each quail received a single session of 8 presentations of a new object. The procedure was similar to the one used during habituation with the exception of the object. As previously, the amount of time spent in the zone close to the novel object was recorded during each object presentation. The novel object was a plastic cube (edge of 40 mm). Each square surface of the cube was of a different colour (red, blue, green, white, etc.).

2.3. Behavioural analysis

All behavioural observations were performed 'blind' with respect to the line. The home cage was sub-divided into two zones, the object zone including the site of object presentation (Fig. 1).

2.4. Statistical analysis

All data were analysed by parametric analyses of variance (ANOVA) with lines (STI vs. LTI) as between-subject factors and with presentations and days as within-subject factors.

For habituation, first, the overall effect of line on the mean time spent close to the object was examined. Then, the effect of presentations and more importantly the effect of days on the time spent close to the object were examined. An increase in the time spent close to the object over days would assess that habituation occurred. Third, we also analysed the evolution over days of the time spent close to the object during the first presentation. Indeed, object habituation in this test is based on novelty detection. It is thus possible to postulate that, each day, the time spent close to the object on the first presentation better reflected how the animal evaluated the novelty of the object.

During dishabituation, first, the overall effect of line on the mean time spent close to the novel object was examined. Second, analyses were conducted to compare the time spent close to the object between the last session of habituation (habituated object) and the session of dishabituation (novel object). If birds detect